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Effects of GnRH Vaccine, Improvac®, on Oestrus, Ovarian Activity and Growth in Japanese Black Fattening Beef Heifers

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INTRODUCTION

Oestrus and mounting behaviors in female beef herds often lead to damage of carcass and reduction of feed intake. Gonadotropin releasing hormone (GnRH) vaccine suppresses gonadal functions in cattle, neutralize biological activity of endogenous GnRH by raising GnRH antibody. This method has been used as chemical castrations of young bulls and cows in several overseas countries. However, there have been no reports regarding effects of GnRH vaccine on gonadal functions of Japanese black fattening beef heifers. The present study was conducted to examine effects of Improvac®, a GnRH vaccine, on oestrus behavior, ovarian activity and growth in Japanese black fattening beef heifers.

MATERIALS AND METHODS

This study was conducted from March 2017 to February 2018. Seven Japanese black fattening beef heifers at 10-11 months of age were used in this study. They were kept in Hyogo Prefecture College of Agriculture for fattening purpose and provided ad libitum hay and concentrate to meet or exceed the Japanese Feeding Standard recommendations for beef heifers. Four out of the seven heifers were treated with a GnRH vaccine, Improvac® (Zoetis Japan, Tokyo; 2 mL including 4 mg) subcutaneously at 10-11 months of age (Week 0). The booster vaccinations were performed at Week 4, Week 20 and Week 36. The other three heifers were not treated and used as a control. The following examinations were performed for 44 weeks from Week 0. Feed intake was examined at every feeding time (twice a day, morning and evening) by subtraction of remained amounts from provided amounts. Body weight was measured once a month. Standing and mounting behaviors were observed every day at twice a day. Palpation of ovaries and uterus per rectum was performed monthly. Blood samples was collected weekly from Week 0 to Week 8 and subsequently Weeks 12, 16, 20, 22, 24, 28, 32, 36, 40 and 44. The blood was taken from jugular vein of beef heifers into

heparinized tubes and immediately placed in ice before centrifuging (1,700 x g for 20 minutes at 4°C). The plasma was stored (-30°C) until the assay. Plasma progesterone concentrations were assayed by EIA. Data were expressed as mean±SEM. We examined the effects of treatment and time, and interaction of both factors by conducting an analysis of variance (ANOVA) using the Generalized Linear Models procedure of SPSS ver. 22 software (IBM, Somers, NY). The differences between GnRH vaccine treatment and control group were examined by conducting pairwise comparisons of the GLM procedure by the least significant difference (LSD) as a post hoc test.

RESULTS AND DISCUSSIONS

Occurrence of standing and mounting behaviors were more frequently in control group than GnRH vaccine treated group, especially after 5-6 months after the second GnRH vaccine (the first booster injection; Table 1). In the control group, corpus luteum was palpated in the ovary until Week 36 after the start of the experiment (Table 2). However, the corpus luteum was palpated until Week 20 after the GnRH vaccine treatment and thereafter no corpus luteum was found in the ovary. In the control group, all three heifers showed cyclic increases of plasma progesterone concentrations over 0.5 ng/mL throughout the experimental period (Fig. 1). In the GnRH vaccine treated group, all four heifers showed cyclic increases of plasma progesterone concentrations over 0.5 ng/mL until Week 24, but after the time until Week 44 the progesterone concentrations were low less than 0.5 ng/mL. The effect of GnRH vaccine treatment was significant ($P < 0.0001$) on weekly changes of plasma progesterone concentrations (Fig. 2). When the progesterone data were divided into "Before (Weeks 0-4 & 16-20)" or "After (Weeks 5-12 & 22-44)" the GnRH vaccine treatment (Fig. 3), the progesterone concentrations were significantly lower ($P < 0.0001$) in the GnRH vaccine treated group than in the control group in the "After" period. Body weight increased significantly ($P < 0.0001$) during

the experimental period and there was no difference between the GnRH vaccine treated and control groups (Fig. 4).

In this study we examined effects of GnRH vaccine, Improvac® on oestrous behaviors, ovarian cyclic activity and growth of Japanese black beef fattening heifers. GnRH vaccine compete with natural GnRH for binding to GnRH receptors, thus suppressing or blocking GnRH action in the body[5]. GnRH is responsible for the release of LH and FSH from the pituitary, to maintain reproductive cycle of follicle in ovary[5]. Suppressing GnRH will lead decreasing of ovarium activity. Progesterone is one of steroid hormone produced by corpus luteum in ovarium, and can be used for indicator of ovarium activity. GnRH antagonist is not a hormone it was a vaccine, biohazard generally save, zero residu of 1 day onset of action[4]. It can be used for alternative immunosterilisation in fattening program or population control[6].

GnRH antagonist Improvac® (Zoetis), was introduced to the market for immunocastration of male pig. A new vaccine Bopriva® designed specifically for cattle, is the only anti GnRH antagonist available in cattle on the market and has demonstrated good efficiency[3]. Improvac® consist of a synthetic GnRH analog coupled with diphtheria toxoid wich act as a larger immunogenic carrier protein for the analogue. Improvac® is also formulated with ionic polysaccharide adjuvant DEAE dextran, but Bopriva® couples DEAE dextran with another adjuvants that are capable of increasing the immune stimulation effect of a vaccine, while decreasing reactogeneticity at other adjuvant present. they do this by binding the other adjuvant thereby creating a new complex with altered reactogenicity [3].

As the results, it is likely that treatment with GnRH vaccine reduced standing and mounting behaviors without effects on growth of heifers. The inhibitory effects on estrus behaviors of heifers were probably achieved by inhibition of ovarian cyclic activity because corpus luteum was not formed and plasma progesterone concentrations were lower than 0.5 ng/mL after Week 24 until the end of the experiment in the GnRH vaccine treated group. It is well known that the GnRH vaccine raises anti-GnRH antibody in the treated animals and neutralize endogenous GnRH, then suppresses LH secretion from anterior pituitary gland. However, in the GnRH vaccine treated group, progesterone concentrations increased Weeks 16 and 20, indicating the inhibitory effect on ovary did not continue over 3 months after the booster injection at Week 4. The progesterone concentrations decreased after the third booster injection at Week 20 thereafter the concentrations were kept low levels until Week 36. These data suggest the inhibitory effects of GnRH vaccine on

ovarian activity can continue only for 3 months. Thus 3 months intervals of the GnRH vaccine treatments may be recommended for complete suppression of ovarian activity and oestrus behaviors in the fattening beef heifers.

Table 1. Occurrence of standing and mounting behaviors in individual Japanese Black fattening beef heifer between GnRH vaccine treatment (A-D) and untreated control (E-G)

Heifers	Months after the second GnRH vaccine									
	1	2	3	4	5	6	7	8	9	10
GnRH vaccine	A	•	•	••					•	
	B	•••	•••				•			
	C		••						••	
	D	•		•					•	
Control	E	•••	•••	•••			•••	••	••	•••
	F	•••	•••	•••	•	••	•••	••	••	••
	G	•••	•••	•••	••	••	•••	••	••	••

Table 2. Ovarian structures in individual Japanese Black fattening beef heifer between GnRH vaccine treatment (A-D) and untreated control (E-G)

Heifers	Weeks after the first GnRH vaccine									
	0	4	8	12	16	20	24	28	32	36
GnRH vaccine	A	—	F	—	F	CL	—	—	—	—
	B	CL	—	—	F	—	—	—	—	—
	C	CL	—	—	F	F	CL	—	—	—
	D	—	—	—	—	—	F	—	—	—
Control	E	—	—	—	×	F	×	—	—	CL
	F	×	×	×	×	×	×	×	—	CL
	G	F	CL	F	CL	F	CL	—	F	CL

CL: Corpus luteum was palpated.
F: Follicle or follicles were palpated.
—: No corpus luteum nor follicle.
×: Ovaries cannot be palpated.

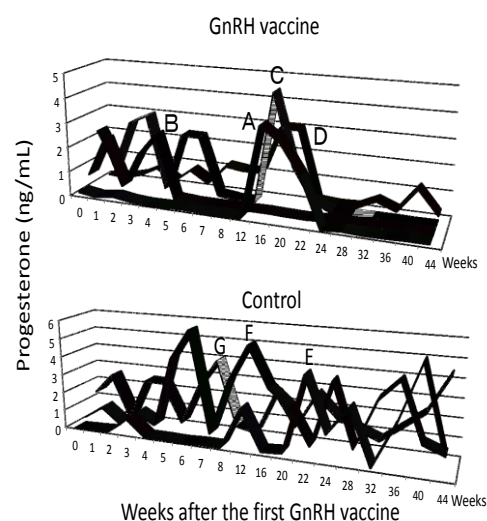


Fig. 1. Changes of plasma progesterone concentrations in individual Japanese Black fattening beef heifer between GnRH vaccine treatment (A-D) and untreated control (G-F) groups

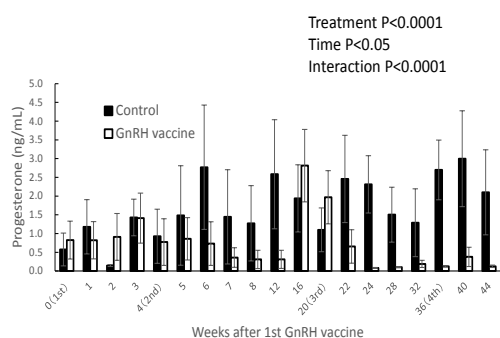


Fig. 2. Effects of GnRH vaccine on changes of plasma progesterone concentrations in Japanese Black fattening beef heifers

Data were expressed as mean \pm SEM.

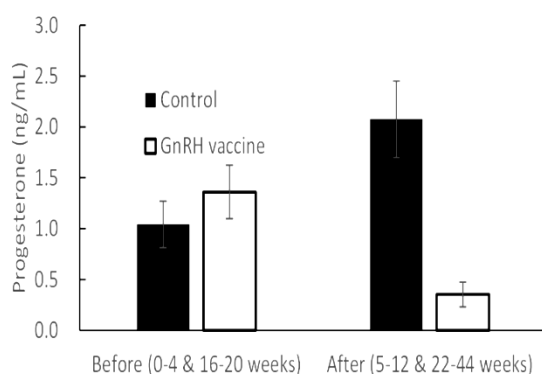


Fig. 3. Effects of GnRH vaccine on progesterone concentrations before and after treatment

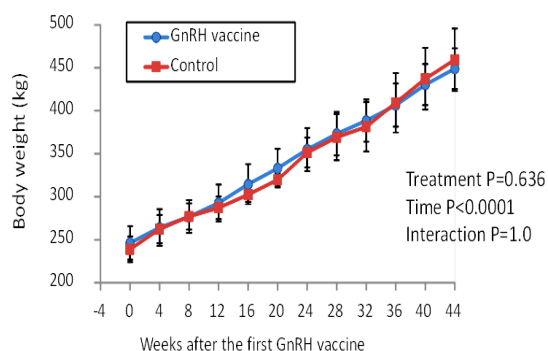


Fig. 4. Changes of body weights in Japanese Black fattening beef heifers between GnRH vaccine treatment and untreated control group

CONCLUSIONS

1. Treatment with GnRH vaccine, Improvac®, in Japanese black fattening beef heifers seems to reduce oestrus behaviors after 6 months of treatment without affecting body weight gain.
2. Cyclic ovarian activity was suppressed for 3 months after the booster injection of the GnRH vaccine.
3. Further studies are required to determine optimal intervals of the GnRH vaccine treatments to suppress completely ovarian activity and oestrus behaviors in the fattening beef heifers.

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